

## Second Quarter Report Public Page

Date of Report: *December 15, 2008*

Contract Number: DTPH56-08-T-000014

Prepared for: DOT-PHMSA

Project Title: Effect of Concentration and Temperature of Ethanol in Fuel Blends on Microbial and Stress Corrosion Cracking of High-Strength Steels

Prepared by: Colorado School of Mines and National Institute of Standards and Technology

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For quarterly period ending: *December 15, 2008*

<b><u>Activities/Deliverables:</u></b>	<b><u>Expected</u></b>	<b><u>Progress</u></b>
	<b><u>Month</u></b> <b><u>Due:</u></b>	<b><u>%</u></b> <b><u>Complete:</u></b>
Task #1: Kick-off Meeting and summary report	2 <sup>nd</sup> month	100 %
Task #2: Receipt of pipeline and tank materials, corrosive media, and biological agents	2 <sup>nd</sup> month	100%
Task #3: Collect literature and data in preliminary report to include in 1st quarterly report	3 <sup>rd</sup> month	100%
Task #4: Setup corrosion cells, calibrate analytical practice and prepare corrosion specimens (in-progress).	33 <sup>rd</sup> month	20%
Task #5: Setup mechanical testing apparatus, machine specimens, and calibrate mechanical testing practices (in-progress).	33 <sup>rd</sup> month	25%
Task #6: Make contacts and plan field visits to analyze actual corrosion (in-progress).	33 <sup>rd</sup> month	55%
Task #7: Submit 1 <sup>st</sup> Quarter Report	3 <sup>rd</sup> month	100 %
Task #8: Interim progress report for calibration of impedance resistivity measurements	9 <sup>th</sup> month	10 %
Task #9: Perform initial corrosion and microbiological corrosion testing on X52 grade pipeline steel and A36 tank material using E100	33 <sup>rd</sup> month	5 %
Task #10: Perform baseline mechanical property measurements on X52 grade pipeline steel and A36 tank material (in progress)	33 <sup>rd</sup> month	5 %
Task #11: Collect microbiological specimens and steel at field sites (in progress)	33 <sup>rd</sup> month	5 %
Task #12: Submit 2 <sup>nd</sup> Quarter Report	6 <sup>th</sup> month	100 %

### **Technical Status Section:**

Technical efforts for the second quarter of the project period included material procurement, development of mechanical testing capabilities and procedures, a field visit with sample collection, development of biological thin-film interfacial (metal substrate) studies, continued biological survivability experimentation, and benchmark (MIC of linepipe steel in petroleum) investigations to demonstrate feasibility of using advanced corrosion analytical tests to assess microbiological corrosion. Mechanical testing efforts included refining testing parameters for the initial phase of the project, designing and fabricating a preliminary submerged bending fixture, and designing a multi-specimen submerged bending fixture. Biological studies included continued biological viability studies in reagent grade ethanol and characterization and testing of field ethanol-sludge samples.

### **Literature Review**

An on-going literature review has continued to be conducted. Three peer-reviewed microbiologically influenced corrosion articles have been written and for publication. These have been uploaded as additional documents along with this report. A review of the current state of knowledge of ethanol stress corrosion cracking has also been drafted and is being edited.

### **Material Procurement and Characterization**

Steel material was acquired and specimens were machined from ASTM A-36 tank steel, API -5L (X70) linepipe steel, and API-5L (X42/52) linepipe steel. Colorado Interstate and Gas (El Paso Corp) provided both grades of linepipe steel. Four feet (1.22 m) of 20-inch (508-mm) diameter 0.260-inch (6.6-mm) Wall Grade X70 ERW with FBE coating and 5 feet (1.52 m) of 12-inch (305-mm) diameter - standard wall - Grade X42/52 with FBE coating were delivered. The Steel Tank Institute through Eaton Steel provided 20 square feet (1.86 square m) of 0.250-inch (6.35-mm) thick ASTM A-36 steel plate.

All components for mixing a standard synthetic fuel grade ethanol (SFGE) were procured. Ethyl alcohol (200 proof, absolute ACS/UPS grade) was attained for preparation of SFGE. Denaturant (natural gasoline) was provided by Merrick and Company. All other components were provided by the Colorado School of Mines.

A practice was developed to characterize steel material. Characterization will include tensile testing, optical and electron microscopy, and chemical analysis. Tensile specimens have been machined. Tensile specimens will be tested to establish material properties for the acquired steel.

### **Preliminary Mechanical Testing**

Four-point bend testing methods defined in ASTM E 399, ASTM E 855 and ASTM C 1161 were studied to refine loading parameters. Four-point bend testing will be loaded at

a cyclic slow strain rate. The mean load will be 75 pct of the material yield stress. Loading will cycle to  $\pm 10$  pct of the mean load. The specimen will be loaded at a rate of  $10^{-6}$  per second and unloaded at a rate of  $10^{-4}$  per second (also per communications with Dr. R. Kane).

Literature review has prompted some refinement of testing variables (Table 1). All specimens will be pre-cracked to aggravate cracking behavior. Denaturant levels will not be varied to better focus efforts on more significant parameters. Dissolved oxygen concentration will be varied. Dissolved oxygen concentration and ethanol cracking exhibit a strong correlation. The availability of dissolved oxygen also determines the types of microbes present. Testing will be conducted in aerated and deaerated conditions to address the strong correlation between oxygen and ethanol cracking. Aeration will be maintained by forced breathing air. Deaeration will be established by nitrogen purging. Methanol, chloride, and sulfate will still be maintained at the upper limits of the ASTM D 4806 specifications for fuel grade ethanol. SFGE water content will still be maintained both at the upper limits of ASTM D 4806 and at an SCC inducing value higher than ASTM D 4806 specification. Synthetic ethanol fuel blends (SEFB) E95, E85 and E50 will still be analyzed. Biological contaminants will be added in some tests to the submerged steel specimen prior to loading. Electrochemical equipment will be set up to monitor solution potential.

Table 1: Mechanical testing variables

Steels	Synthetic Fuel Grade Ethanol (SFGE)	Synthetic EtOH Fuel Blends	Aeration
1) X52 2) X70 3) A36	1) ASTM upper limits 2) ASTM upper limits w/ high water content	1) E95 2) E85 3) E50	1) aerated 3) deaerated

Adaptors have been designed and machined to connect a four point bending fixture to the servo-hydraulic MTS 20 kip loading frame located in the NIST mechanical testing laboratory in Boulder, Colorado. A steel bath to encase the bending fixture and hold corrosive media has been designed and is being fabricated.

#### Multi-Specimen Mechanical Testing

To compensate for the relatively long periods required to run mechanical testing using selected loading parameter, options for increased testing capacity were explored. Preliminary designs were developed for multi-specimen loading fixtures. The proposed design depicted in Figure 1 was selected.

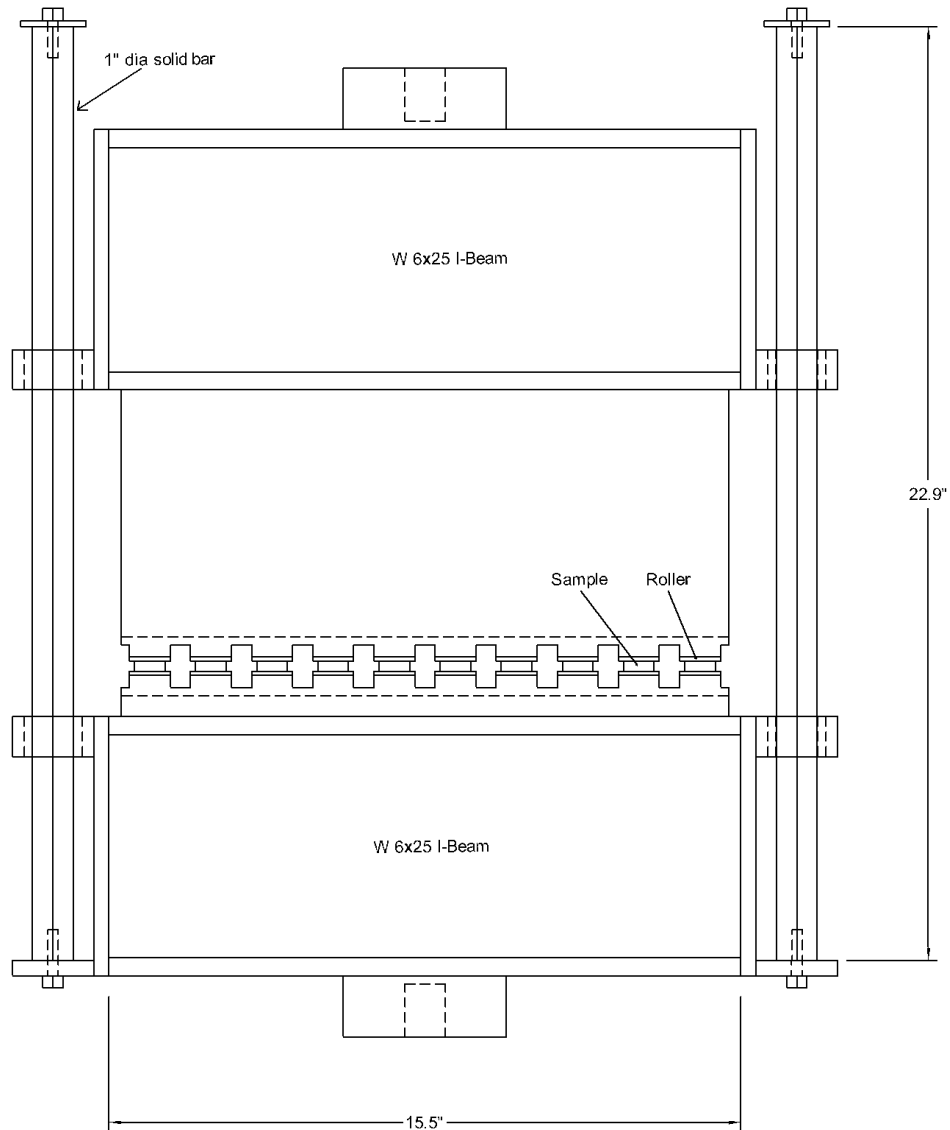


Figure 1: Design for Multi-Specimen Fixture (bath not shown)

The fixture will be mounted on the servo-hydraulic MTS 20 kip loading frame located in the NIST mechanical testing laboratory in Boulder, Colorado. The proposed design will incorporate ten individual specimen mounts. This design will dramatically increase data generation for each test run. The bath and mounts will be supported and held rigid by two steel I-beams (W6x25). The I-beams will be ground flat to insure uniform compressive loading. All material in contact with corrosive media including the mounts, rollers, and bath will be fabricated out of stainless steel. The two I-beams will be aligned by two 1-inch (25.4 mm) diameter guides. A floating cover will minimize ethanol evaporation.

Further Testing at NIST:

Future experimentation by NIST will consist of mechanical testing using compact tension (CT) specimen according to the procedures in ASTM E 647.

### Thin Film Studies

Experiments are being designed to grow microbes in a nanolayer of adsorbed water along a metal and ethanol solution interface. A very thin layer of iron will be deposited on an optical glass slide that will be inoculated with microbes. Optical microscopy directed through the glass plate and thin transparent iron coating will be used to exam the presence of microbial activity associated with the Helmholtz double layer adhering to the metal surface. Variables to be studied are changes in water, fuel, metal cation, and anion concentrations in the ethanol. Iron targets have been ordered to deposit a thin iron film on optical slides using a chemical deposition laboratory at the Colorado School of Mines.

### Field Contacts and Visits

A site visit to an ethanol production facility was conducted to collect samples from an ethanol storage tank. Tank bottoms samples (Figure 2) were collected aseptically and preserved for analysis via molecular biology techniques including DNA sequencing.



Figure 2 – (Left) View looking into an ethanol storage tank from which bottoms samples were collected. (Right) View of collected bottoms sample.

Arrangements for sample collection have been made with a second ethanol production facility as well. A sample from the storage tank filter will be collected upon filter replacement. Ethanol and oil industry representatives continue to be contacted to arrange other sampling opportunities. Ethanol/sludge samples from industry infrastructure will provide information about microbial communities that may be present in these systems and what roles these microbes may play in corrosion.

### Molecular Biology Laboratory Efforts

Molecular biology tools have been implemented to provide evidence of biological activity in fuel-grade ethanol infrastructure. Attempts were made to analyze the tank

bottoms sample collected from the ethanol production facility via 16S rRNA gene sequencing. High iron content (a potential corrosion product) interfered with DNA extractions and subsequent PCR reactions, which are two important processes in the sequencing method. Alternative DNA extraction techniques have been performed to extract DNA from the sample. Preliminary results have suggested that a technique involving magnet separation of iron particles followed by pre-cell-lysis EDTA washes has successfully extracted DNA from the sample. The extracted DNA has been used for preliminary 16S rRNA gene sequencing. The extraction technique and 16s rRNA sequencing results are currently being verified.

### Biological Viability Studies in Ethanol

Preliminary biological viability studies were conducted to ascertain the ability of microbes to survive exposure to 100 pct ethanol. A small amount of soil was placed in 100 pct ethanol for 39 days. Luria broth agar plates were inoculated with this mixture and incubated at 37 °C for 12 hours. Some microbes were able to survive the exposure to ethanol and grow on the culture media. The resulting colonies were identified by 16S rRNA gene sequencing. Preliminary results indicate that all identified colonies fall into the *Bacilli* class of Bacteria.

Members of the *Bacilli* class are known spore-formers, which could explain their ability to survive exposure to 100 pct ethanol. The ability of microbes to survive exposure to ethanol may have implications in systems experiencing batch-flow operation. Interestingly, *Bacillus* species have also been associated with biofilm formation as well as iron and manganese oxidation.

Cultivation experiments are being designed to investigate microbial activity on steel coupons in varying concentrations of ethanol. These experiments will continue to assess microbe survivability in ethanol as well as investigate microbial effects on the steel surface.